Lesson Plan for Biology: Chromatography of Plant Pigments

Objectives:

Students will be able to

- Understand what molecules make living leaves green and autumn leaves multicolored
- Be able to perform a chromatographic experiment to separate a mixture of pigments
- Be able to explain how chromatography works and how one identifies each compound

California Content Standards:

- Grade 7, Physical Principles in Living Systems, 6b & 6f
- Grades 9 - 12, Chemistry, 6f

Background:

Photosynthesis is the process plants use to convert carbon dioxide and water into carbohydrates using the energy of light.

$$\text{Light} + CO_{2(g)} + H_2O_{(l)} \rightarrow C_6H_{12}O_{6(aq)} + O_{2(g)}$$

Special plant chemicals called pigments absorb light at specific wavelengths and use the energy to split water. As the water splits into oxygen and hydrogen ions, electrons are released and begin the photosynthetic path that eventually leads to the formation of the simple sugar glucose.

$$2\ H_2O_{(l)} \rightarrow O_{2(g)} + 4\ H^+$$

The main photosynthetic pigments are chlorophylls that give the green color to plants. They often hide the other pigments that may be present. Chlorophyll a is a bright green to blue green color and chlorophyll b is a yellow to olive green to the human eye. This is the color that is reflected by the pigment. All other colors are absorbed especially reds and blues. In addition to chlorophyll many green plants contain one or more other pigments including the yellow/orange carotenes, yellow xanthophylls, ands red to purple anthocyanins.

Chromatography is a separation technique invented in 1910 by the Russian botanist Mikhail Tswet for the purpose of separating plant pigments. The word chromatography is derived from the Greek words for color (chromo) and writing (graph). Chromatography has a stationary phase such as paper, or a thin layer of an absorbent substance and a mobile phase, the solvent that will dissolve the pigment.

Purpose:

To separate the pigments contained in spinach leaves.

Materials and Supplies:

- Chromatography paper strips
    - Whatman® #1 about 1 cm by 15 cm
- Coffee filter or filter paper
- Capillary tube or coffee straws
- Solvent (92% petroleum ether, 8%Acetone)
    - Acetone may be used alone if necessary
- 50 mL beaker or small jar
- 250 mL beaker or tall glass jar
- Aluminum foil
- Rubber bands
- Goggles and gloves
- Metric ruler

Prelab prep:

The day before the lab tear fresh spinach leaves and place them in a glass container. Cover the leaves with acetone to extract the pigments. Be sure to cover the glass container to keep the acetone from evaporating.

HAZARD: Acetone and the developing solutions are flammable. Keep them away from sparks and open flames. Always wear goggles.

Chromatography Procedure:

1. Pour the acetone-spinach solution through a filter and collect the liquid in a 50 mL beaker.

2. Wear gloves when handling the chromatography paper strips to keep the oils of your skin off the paper. Draw a pencil line about 1.5 cm from the end of your paper strip.

3. Use a capillary tube coffer straw to place a small drop of your green solution on the center of the line.

4. Be sure to let the drop completely dry before you add another drop. This will help keep the spot from spreading out too much. Repeat 15 to 20 times. Be careful not to let the spot spread out too far.

5. Pour enough chromatography solution into the 250 mL beaker to completely cover the bottom.

6. Stretch the rubber band around the 250 mL beaker lengthwise over the mouth and bottom if the beaker. This will provide a place to hang the chromatography strip.

7. Suspend the paper strip in the beaker by hanging it over the rubber band. You can attach it with a paper clip or simply fold the end of the strip over the rubber band.

8. The end of your strip should just touch the solvent.

9. Cover the beaker carefully with aluminum foil.

10. Observe as the pigments travel up the paper strip. After about 20 to 30 minutes remove your developed chromatogram. Be careful to handle the paper by the paper clip or top fold only.

11. Quickly before it dries, mark with a pencil where the solvent stopped. This is called the solvent front.

12. Also mark where each pigment stopped moving.

Data:

What observations would help you analyze your chromatogram? Construct a data table to organize these observations.

Analysis:

The relationship between the distance the pigment moved to the distance the solvent moved is called the R_f value.

$$R_f = \frac{\text{Distance traveled by the pigment from the origin}}{\text{Distance traveled by the solvent from the origin}}$$

Calculate the R_f values for each of your pigments.

Conclusions:

1. Which pigments were you able to observe in your chromatogram? How did you determine you actually had these particular ones?

2. Why do the pigments move at different rates through the chromatogram?

3. Why is it an advantage for plants to have different colored pigments?

4. With what you have discovered about plant pigments, what conclusions can you make regarding the changing color of leaves in autumn?

5. Think of a new experiment you would like to try using the new skill you have learned.